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Post-renal Transplant Thrombotic Thrombocytopenic Purpura (TTP): Attributable to Immunosuppression or Graft Rejection?

Report of Three Cases and literature review

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ABSTRACT:

The immunomodulatory drug FK-506 (Tacrolimus) is a calcineurin inhibitor that, as such, indirectly inhibits the transcription of a gene encoding interleukin 2, a cytokine that is vital to the immune response process. Since its introduction in 1987, sporadic reports have appeared describing the development of thrombotic thrombocytopenic purpura (TTP) - or a similar syndrome - in patients taking this drug. Sirolimus (Rapamune) is another immunomodulatory drug with a more favorable side-effects profile than calcineurin inhibitors. We report three cases of TTP. The first case is of a 28-year-old woman who developed severe abdominal pain, a precipitous drop in platelet count, increase in serum lactate dehydrogenase and a coombs-negative hemolytic anemia, all of sudden onset, three weeks after undergoing a kidney transplantation. Post-transplant immunomodulation was with both Tacrolimus at 4mg p.o. bid, and Sirolimus at 10mg p.o. qd; there were no signs of renal dysfunction at presentation. Tacrolimus was substituted with Cyclosporin and daily plasma exchanges resulted in near normalization of clinical and laboratory parameters. The patient relapsed 10 days after discharge and Cyclosporin was replaced with mycophenolate mofetil; She had no further relapses at 4.5 months follow-up. The second case is of a 57 year old African American female with ESRD secondary to arteriosclerotic disease (history of CAD, hypertension , Diabetes mellitus and hyperlipidemia). She had no difficulties with the transplant and was discharged with Rapamune (sacrolimus), Prograf, and prednisone. Seven days after transplant she had increased edema and decreased renal function. Biopsy revealed humoral rejection. Apheresis called to perform TPEx. Patient also noted to have shistocytes, elevated LDH, decreased platelets. The patient received 5 consecutive days of TPEx with cryopoor FFP to treat both acute rejection and TTP. Thymoglobulin was started and other rejection medications were held to treat the rejection and aid in resolution of the TTP. There was no improvement clinically or in laboratory values. She had explantation 16 days after transplant. At time of discharge the platelet count was in normal range and the LDH level

was much improved. There is no recurrence in the past 1.5 years. The third case is of a 50 year old African American female with a history of acute humeral rejection of a renal transplant for ESRD (secondary to idiopathic nephrotic syndrome). She presented for DDRT and 10 days following developed acute rejection proven by percutaneous biopsy. The acute humoral rejection was treated with TPEx, high dose IVIG and tacrolimus. At start of initial apheresis she was noted to have dark brown plasma. TTP was considered. Platelet count was 50K/microL, LDH 765U/L, HCT 20%, haptoglobin was <6 mg/dL, shistocytes were on the peripheral smear and DAT was negative. The patient's TTP and acute rejection resolved after 20 treatment of TPEx with cryopoor TTP. At discharge she was on Tacrolimus, Cellcept and oral steroids. There has been no recurrence 4 months after completion of plasmapheresis course.

Thrombotic thrombocytopenic purpura can be induced with medications to prevent rejection or with rejection itself. Direct *causality* is not entirely demonstrable in cases involving FK-506 (Tacrolimus) or Sirolimus (Rapamune) use. However, these *associations* should be highlighted whenever encountered so that it can be determined whether the incidence of FK-506/Sirolimus associated TTP exceeds that of the general population – i.e. whether or not these associations are *coincidental*. Previously reported cases of FK-506 and Sirolimus associated TTP are also summarized and reviewed.

ABSTRACT:

The immunomodulatory drug FK-506 (Tacrolimus) is a calcineurin inhibitor that, as such, indirectly inhibits the transcription of a gene encoding interleukin 2, a cytokine that is vital to the immune response process. Since its introduction in 1987, sporadic reports have appeared describing the development of thrombotic thrombocytopenic purpura (TTP) - or a similar syndrome - in patients taking this drug. Sirolimus (Rapamune) is another immunomodulatory drug with a more favorable side-effects profile than calcineurin inhibitors. We report three cases of TTP. The first case is of a 28-year-old woman who developed severe abdominal pain, a precipitous drop in platelet count, increase in serum lactate dehydrogenase and a coombs-negative hemolytic anemia, all of sudden onset, three weeks after undergoing a kidney transplantation. Post-transplant immunomodulation was with both Tacrolimus at 4mg p.o. bid, and Sirolimus at 10mg p.o. qd; there were no signs of renal dysfunction at presentation. Tacrolimus was substituted with Cyclosporin and daily plasma exchanges resulted in near normalization of clinical and laboratory parameters. The patient relapsed 10 days after discharge and Cyclosporin was replaced with mycophenolate mofetil; She had no further relapses at 4.5 months follow-up. The second case is of a 57 year old African American female with ESRD secondary to arteriosclerotic disease (history of CAD, hypertension , Diabetes mellitus and hyperlipidemia). She had no difficulties with the transplant and was discharged with Rapamune (sacrolimus), Prograf, and prednisone. Seven days after transplant she had increased edema and decreased renal function. Biopsy revealed humoral rejection. Apheresis called to perform TPEx. Patient also noted to have shistocytes, elevated LDH, decreased platelets. The patient received 5 consecutive days of TPEx with cryopoor FFP to treat both acute rejection and TTP. Thymoglobulin was started and other rejection medications were held to treat the rejection and aid in resolution of the TTP. There was no improvement clinically or in laboratory values. She had explantation 16 days after transplant. At time of discharge the platelet count was in normal range and the LDH level

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INTRODUCTION:

The term thrombotic thrombocytopenic purpura (TTP) was introduced into the medical lexicon by Moschowitz (1) in 1925 in a case report describing fever, anemia, central nervous system impairment, renal dyspunction and cardiac failure in a 16-year-old female. Hyalin thrombi in the terminal arterioles was considered characteristic of the disorder. Thirty years later, Gasser et al (2) described 5 children with hemolytic anemia, thrombocytopenia and acute renal failure and introduced the term hemolytic-uremic syndrome (HUS). HUS is generally a disease of children associated with hemorrhagic colitis following infection with Shigella toxin-producing strains of Shigella bacteria (e.g *Shigella dysenteriae*) or *Escheria coli* (e.g O157:H7). In adults however, there has been an increased tendency over the past few decades to view HUS and TTP as different manifestations of the same disease process, with the former term referring to cases in which renal signs predominate and the latter term referring to those in which neurological signs predominate (3). This tendency stems from the overlap in clinical features, the commonality in therapeutic modalities and the difficulty in distinguishing based on laboratory parameters between the two syndromes (3). An estimation of only one in six patients with TTP/HUS presents with the classic pentad of thrombocytopenia, microangiopathic hemolytic anemia, fever, renal failure and neurological anomalies (4). Since plasma exchange can be life-saving, decreasing mortality from 80-90% to 10-20%, diagnostic criteria for this syndrome have been loosened by necessity. A patient presenting with coombs-negative hemolytic anemia, severe thrombocytopenia and a significant

increase in serum lactate dehydrogenase that is unexplained by other factors is considered as having TTP/HUS until proven otherwise (4)

Endothelial damage is believed to be the central and underlying event in this syndrome (3). This endothelial damage may be precipitated by known agents or clinical conditions, e.g viral infection, pregnancy or post-partum status, solid organ or bone marrow transplantation, pancreatitis, malignancies, IgA nephropathy, or drugs (secondary TTP/HUS). Alternatively TTP/HUS may stem from a congenital anomaly in the activity of compounds such as von Williebrand factor-cleaving metalloproteinase or Factor H (Familial TTP/HUS) (3). Finally, as is true in most cases, the underlying etiology may be largely unknown, i.e idiopathic TTP/HUS (3).

TTP has also been reported to be associated with post organ transplant as well as allogeneic BMT. The changes seen in BMT are similar to those of TTP. Difficulty lies in distinguishing between either. The changes seen in BMT may be a common final pathway to many inciting agents. Plasmapheresis is not beneficial in these patients. Changes in vWF cleaving protein are not seen in these patients.(34) Treating with removal of offending drug is beneficial. (32) Post-Organ transplant thrombotic microangiopathies have been seen following kidney, liver, heart, lung pancreas and intestine. Most post transplant TTPs have been attributed to the use of immunosuppression drugs (e.g. Cyclosporin A{CSA}, tacrolimus{FK506}). (31) Immunosuppressive agents have been shown to have direct toxicity to endothelial cells. (33) All have been reported to be associated with normal vWF levels.(31) It also believed that other factors may

also increased the risks of post transplant TTP including endothelial damage by transplant procedure or immunosuppressive agents. (31) A two hit hypothesis is supposed with damage to endothelial cells from cold ischemic time, reperfusion injury, sensitization and vascular rejection as being the first hit with immunosuppressive agents as the second hit. (33) After initial TMA is resolve restarting immunosuppressive agent typically does not lead to recurrence of the TTP. (33)

In particular, transplant rejection in the kidney is often accompanied by shistocytes, elevated LDH and microthrombi demonstrated on biopsies. (31) The acute rejection can occur with the TTP. (33) Treating acute humoral rejection with apheresis may be adjunctive to modifications in medical regimens. Well-documented cases are present with continued TTP even with only stopping immunosuppressive agents. (33)

Here we report three cases of post renal transplant TTP, one associated with the combined use of tacrolimus (FK506) and sirolimus (Rapamune) and two with evidence of acute humoral vascular rejection. We review the relevant literature on the subject.

CLINICAL HISTORY:

Patient 1

The first patient is a 25-year-old woman with end-stage renal disease (ESRD) due to focal segmental glomerulosclerosis received a renal transplantation

donated by a 31-year-old male sibling. The immediate post-operative course was devoid of complications with hourly urine output of approximately 200 cc/hour on post-operative day (POD) 1. In addition, post-operative blood urea nitrogen and serum creatinine levels dropped to 14 mg/dl and 1.2 mg/dl from pre-operative levels of 58mg/dl and 5.5mg/dl respectively. Excellent graft function was present. The patient's post-operative immunosuppressive regimen consisted of Prednisone 30mg q6 on POD 1, was tapered down to 15 mg tid by POD 5. Sirolimus 15 mg p.o. on POD 0, was tapered to 10 mg p.o. bid on POD 2, then to 5 mg p.o. qd on POD 3. Tacrolimus 2mg p.o. was started on POD 2. The patient was discharged on POD 5. On POD 24, the patient developed nausea, sharp, non-radiating epigastric pain and was advised to go to the emergency room. Vitals signs were afebrile and stable with a slightly elevated pulse of 102/minute. A physical examination revealed mild conjunctival icterus, tenderness in the epigastric region and the right upper quadrant. She also reported red micturation. She was found to have a platelet count of 13 x 10^3/ΠL, LDH of 1570 U/L, HCT 25% and total bilirubin of 2.04 mg/dl. A peripheral blood smear showed multiple schistocytes in almost every high-power field, as well as multiple polychromatophilic erythrocytes, ovalocytes, teardrop cells and poikilocytes. Her DAT was negative. Spot urinalysis revealed red-colored urine with only 2-5 red blood cells per high power microscopic field; Myoglobulin and nitrites were negative. A presumptive diagnosis of thrombotic thrombocytopenic purpura was rendered. Tacrolimus was discontinued and replaced with Cyclosporin (75 mg each morning and 50 mg each evening), and the plasma exchange was initiated.

Over the subsequent 9 days, the patient underwent 9 daily plasma exchanges. She showed significant clinical improvement, as manifested by cessation of abdominal discomfort, progressive increase in her platelet counts from a nadir of 11,000/ul to 128,000/ul, and a significant decrease in her serum lactate dehydrogenase (LDH) from a peak of 1570U/L to 253 U/L. The patient was discharged on hospital day 9 and was managed as an outpatient. Nine days after discharge, she was re-admitted with a platelet count of 80,000/ul, a serum LDH of 844U/L, a haptoglobin level of <6.0mg/dl (reference range 20-200mgldl), consistent with a relapse. She underwent 4 daily plasma exchanges over this 5-day admission, with serum LDH decreasing to 245U/L by Day 5. Her platelet count, however, showed no significant change and was at 77,000/ul on Day 5. On the first day of this admission, her cyclosporin was replaced with Mycophenolate mofetil. Throughout her entire clinical course (including previous admissions), serum levels of Sirolimus, Tacrolimus or cyclosporine, when measured, were consistently within the prescribed therapeutic ranges. Also of note during this admission, von Willebrand antigen multimers, evaluated on day-2 plasma (after 1 plasma exchange) showed all multimers to be present.
In addition, Von Williebrand factor protease - also measured prior to plasma exchange of the second course of TTP showed normal activity. She was discharged on Day 5 on an immunosuppressive regimen that consisted of Mycophenolate mofetil 1g po bid, Sirolimus 9 mg qd and Prednisone 15 mg po qd. She has been followed as an outpatient over the past 16.5 months and has had no further relapses.

Patient 2

The second patient is a 57 year old African-American female with ESRD (for 7 years before transplant) due to arteriosclerotic disease from diabetes mellitus and hypertension. She received cadaveric renal transplantation. The immediate post-operative course was complicated by delayed graph function. She was discharged on Sirolimus 5 mg/d, tacrolimus 4mg q12h. From admission to discharge the creatinine level decreased from 12.7 mg/dL to 5.3 mg/dL. The BUN rose from 60 mg/dL to 88 mg/dL in the same time period. Eleven days after surgery she was noted to have anasarca with rising creatinine of 7.7 mg/dl. On admission her vitals sign were stable and she was afebrile. The only focal findings were minimal serous secretions at previous surgical site. She was treated with high dose steroids, high dose IVIG, thyroglobulin, held other immunomodulating agents and TPEx begun for presumptive acute rejection had a renal biopsy performed. At the time of first plasmapheresis her LDH was 1870 U/dL, with a hematocrit of 20.8%, haptoglobin<6 mg/dL, platelet count of 75 x 10^{3}/ΠL, with shistocyte seen on peripheral smear and a diagnosis of TTP was made along with acute rejection. TPEX started on POD 13. A von Willebrand Factor Antigen level obtained before plasmapheresis was initiated revealed all multimers present with only a slight decrease in activity of von Willebrand Factor Protease level. On POD 18, after 5 consecutive days of plasmapheresis, her platelet count had decreased further to 26 x 10^{3}/ΠL day a LDH of 961 U/dL with continued shistocytes and a HCT 0f 25% despite transfusion of multiple RBCs. By immuno-histocompatibility testing she was found to have a strong positive

crossmatch for both T and B cells with 80-100% killing of donor lymphocytes by recipient serum. Renal biopsy results were read as thrombotic microangiopathy. At this time she had an episode of acute drop in blood pressure with decrease level of HCT. On ultrasound she was found to have a large perinephric flow collection. On exploration a single hole in the inferior pole of the kidney was visualized. Given no improvement in signs or symptoms from TTP and humoral rejection with inability to likely tolerate further plasmapheresis a nephrectomy was performed. One day after surgery a dramatic improvement in laboratory values was observed. LDH decreased to 464 U/dL with a platelet increase to 78 x 10^3/ΠL. She was discharge 8 days later and received dialysis 3 times per week. At one-month follow-up her Platelet count was 352 x 10^3/ΠL. No relapse has occurred in the last 18 months.

Patient 3

The third patient is a 50 year old African American female with a history of ESRD secondary to focal segmental glomerulosclerosis. She received a transplant in1996 and lost function secondary to acute humoral rejection. She present for second deceased donor renal transplant. Serologies preoperative show no reactions to donor T or B cells. In he immediate postoperative period she had delayed renal function. By POD 5 her renal function peaked then started to decline. POD 10 renal function continued to deminish. A renal biopsy was performed and she was treated for potential acute rejection. Renal biopsy was positive for acute humoral rejection with C4d deposition demonstrated in

peritubule vasculature by immunoflourescence. She was treated with TPEx with 5% albumin, high dose IVIG, Thymoglobulin, Cellcept and prednisone were started for the treatment of acute humoral rejection. Sirolimus was stopped and no additional doses after first dose of tacrolimus were given. She had panel reactive antibodies performed. She had 98% Class I (A24) and 31% Class II (DR 4) present. These antigens were present on the donor kidney. Post Op 11 day she was found to have brown colored plasma during plasmapheresis consistent with TTP. The HCT was 20%, platelet count was 50x 10^3/ΠL, shistocytes were seen on the peripheral smear, DAT was negative, haptoglobin was <6 mg/dL, and a LDH 765 /dL was found. A diagnosis of TTP was made, she received 18 days of TPEX, and her TTP and rejection resolved. During treatment a repeat renal biopsy revealed resolving rejection and thrombotic microangiopathy. Platelets count nadir of 50x 10^3/ΠL and 144 x 10^3/ΠL at discharge with LDH peak of 874 U/dL and 217 U/dL at discharge. Creatinine post treatment was 1.6 mg/dL (peak of 5.1 mg/dL) off dialysis. Four months post discharge no relapse of TTP or rejection has occurred. Discharge medications include steroid taper, tacrolimus and Cellcept.

Figure.

DISCUSSION:

In 1971, Liu et al (5) described the development of microangiopathic hemolytic anemia, renal injury and thrombocytopenia in 3 patients being treated with mitomycin for advanced stage carcinoma. Since that report, an ever-increasing list of medications have been associated with TTP/HUS, with a recent review (6)

listing 53 drugs and other products ranging from oral contraceptives to anti-neoplastic drugs. The strength of these associations among the various medications is highly variable, and the bulk of the literature consists of small series and anecdotal case reports which, similar to ours, describe the development of TTP/HUS in patients taking any of these medications. Medications such as Mitomycin and Cyclosporin have the strongest association because large numbers of cases have been described. For the other medications however, a collective attempt must be made in the medical community to vigorously document and report this association whenever it is encountered, as it is currently unclear whether these associations are merely coincidental. The immunomodulatory drug Tacrolimus (Prograf®, Fujisawa USA, Inc, Deerfield, IL) is emerging as another one of those medications, as experience with the development of TTP in patients taking this drug accumulates. Tacrolimus was initially extracted from culture filtrates of *Streptomyces tsukubaensis* which were themselves discovered from soil samples taken from Mt Tsukuba outside of Tokyo (Japan) in 1984. It was first used in a clinical transplant setting in 1989 and was approved by the Food and Drug Administration (FDA) of the United States in 1994. The mechanism of action of Tacrolimus is not entirely understood. Currently, they are thought to modulate the immune response by inhibiting the activity of an enzyme called calcineurin. The latter is a cytoplasmic enzyme with serine/threonine phosphatase activity whose dephosphorylation of another factor normally results, indirectly, in the transcription of the interleukin 2 gene and consequently the production of interleukin 2 – a requisite for T cell

activation (7). Tacrolimus-mediated inhibition of calcineurin negates the whole cascade and is thus of use in post-transplant settings, for example, where modulation of the immune response is of paramount importance.

The reported cases of FK506 associated thrombotic microangiopathy-TMA - [an inclusive term that describes the morphologic changes of the microvasculature in both HUS and TTP patients (3)], are summarized in Table 2 (8-22). The estimated incidence of Tacrolimus-associated TTP/HUS is 1-4.7% (23). As shown in Table 2, the onset of disease is generally within one year of the institution of therapy. Most of the patients described in these reports have had a favorable outcome following plasmapheresis, a reduction in dosage or discontinuation of Tacrolimus. Other therapeutic modalities that have been somewhat efficacious include anticoagulation therapy and steroids, although, to our knowledge, no randomized, controlled studies exist. It should be emphasized that the analysis of the reported cases is significantly hampered by definitional variations of TTP/HUS or TMA. Most studies only required a decrease in platelet count, microangiopathic hemolytic changes on a peripheral smear and an increase in LDH that is otherwise unexplained by other factors to make the diagnosis of TTP/HUS. Other studies required a biopsy. In one of the latter studies, medication-associated TMA was biopsy-proven in 26 patients. However, the aforementioned derangements in laboratory parameters were only observed in 2 patients (22)

Sirolimus (Rapamune®, Wyeth-Ayerst Laboratories, Radner, USA) is another inhibitor of IL-2-dependent T-cell proliferation that was approved by the

FDA in 1999. Its mode of action involves the formation of an active complex with the cytosolic protein FKB12; the resultant complex inhibits the so-called Target of Rapamycin, which may be involved in the G1 to S phase progression in the cell cycle of eukaryotic cells (24). Sirolimus is generally used in combination with another immunosuppressive medication, and usage in combination with calcineurin inhibitors have been under recent investigation (25-27). *In vitro*, combined usage of Tacrolimus and Sirolimus have a synergistic inhibition of IL-2-mediated lymphocytic proliferation, with the combined effect exceeding that of each individual drug (25). In vivo, the data on this combined usage in the transplant setting is conflicting with respect to side-effect profile and graft survival (26,27). When a patient taking the combination of Sirolimus with another medication develops signs and symptoms of TTP/HUS (28-30), it is impossible to ascertain whether the inducing agent(s) is the combination, or one of the individual drugs. In the current patient, TTP/HUS occurred while she taking both Sirolimus and Tacrolimus, and as outlined above, TTP/HUS association with Tacrolimus, in contrast with Sirolimus, is well-documented in the literature. However, since Sirolimus was approved half a decade after Tacrolimus, it can be expected that the accumulated experience with TTP/HUS-association is limited. It is thus premature to exclude the probability that Sirolimus was partially or wholly responsible for this patient's condition.

The pathogenesis of Tacrolimus or Sirolimus-induced TTP/HUS is currently unclear. Burke et al (20) demonstrated increased levels of several cytokines, including IL10, IL12, tumor necrosis factor-alpha and interferon-

gamma associated with Tacrolimus-associated microangiopathy in the post-transplant setting. The authors postulated that increase in the shear force of the microvasculature (which results in the fragmentation of red blood cells) is attributable to the alterations in the endothelial cells that are mediated by these increased cytokines. Based in part on the observed vasoconstrictive effect of tacrolimus on the renal vasculature, Trimarchi et al (16) speculated that tissue hypoxia, endothelial cell damage and deposition of platelets/fibrin in the glomeruli is a resultant effect.

Lichtman initially notes the association of rejection with post renal transplant TTP in 1968. (35) One very recent retrospective study reported the incidence of thrombotic microangiopathy following renal transplant as being 5.6 episodes per 1,000 person years. Increased risk with younger recipients, older donor, female and initial use of sirolimus at time of discharge. Most episodes occurred within 3 months of transplant date. Patient survival at 3 years was 50%. (36) A prospective study revealed an 80% remission rate of thrombotic microangiopathy after starting plasmapheresis and stopping calcineurin inhibitors. TTP typically occurred within 7 days of transplant. Of the patient placed back on calcineurin inhibitors 95% did not have recurrence of TTP. (33) The episodes of TTP in our patients occurred in 10, 11 and 24 days. TTP resolved in each case by medical management and TPEX with one case requiring explantation to resolve the episode.

In summary, we have documented 3 cases of TTP/HUS associated with renal transplant and Tacrolimus/Sirolimus use. We encourage physicians to

report these associations whenever encountered, to rule out the possibility that these associations are merely coincidental. Future studies should also evaluate the role of therapeutic plasma exchange in treating acute humoral rejection with/without concurrent thrombotic microangiopathy.

REFERENCES

1. Moschcowitz E. Acute febrile pleiochromic anemia with hyaline thrombosis of terminal arterioles and capillaries: An undescribed disease. Arch Intern Med 1925;36:89-93
2. Gasser C, Gautier E, Steck A, et al. Hamolytisch-uramische syndromes bilaterale nierenrindennekrosen bei akuten erworbenchen hamolytischen anamien. Schweiz Med Wschr 1955;85:905-909
3. Ruggenenti P, Noris M, Remuzzi G. Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. Kidney Int 2001;60:831-846
4. Baeyer HV. Plasmapheresis in thrombotic microangiopathy-associated syndromes: review of outcome data derived from clinical trials and open studies. Ther Apheresis 2002;6:320-328
5. Liu K, Mittleman A, Sproue E, et al. Renal toxicity in man treated with mitomycin C. Cancer 1971;28:1314-1320
6. Medina PJ, Sipols JM, George JN. Drug-associated thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Curr Opin Hematol 2001;8:286-293

7. Diasio RB, LoBuglio AF. Immunomodulators: immunosuppressive agents and immunostimulants. In: Hardman JG, Limbird LE, eds. Goodman & Gilman's the pharmacological basis of therapeutics. 9th Ed., McGraw Hill: New York. Pp 1291-1308.

8. Schmidt RJ, Venkat kk, Dumler F. Hemolytic-Uremic Syndrome in a renal transplant recipient on FK 506 immunosuppression. Transplant Proc 1991;23:3156

9. Ichihashi T, Naoe T, Yoshida H, Kiyoi H, Fukutani H, Kubo K, Yamauchi T, Saito H, Ohno R. Hemolytic uraemic syndrome during FK506 use. The Lancet 1992;340:60-61

10. Holman M, Gonwa T, Cooper B, et al. FK506-associated thrombotic thrombocytopenic purpura. Transplantation 1992;22:205

11. Japanese FK506 Study Group. Morphological characteristics of renal allografts showing renal dysfunction under FK506 therapy: is graft biopsy available to reveal morphological findings corresponding with FK506 nephropathy? Transplant Proc 1993;25:624

12. Randhawa P, Tsamandas A, Magnone M, et al. Microvascular changes in renal allografts associated with FK506 (tacrolimus) therapy. AM J Surg pathol 1996;20:306

13. Mach-Pascual S, Samii K, Beris P. Microangiopathic hemolytic anemia complicating FK506 (tacrolimus) therapy. Am J Hematol 1996;52:310

14. Katari S, Magnone M, Shapiro R, Jordan M, Scantlebury V, Vivas C, et al. Clinical features of acute reversible tacrolimus (FK506) nephrotoxicity in kidney transplant recipients. Clin Transplant 1997;11:237

15. Garber L, Moore L, Reed L, et al. Renal histology with varying FK506 levels. Transplant Proc 1997;29:186

16. Trimarchi HM, Truong LD, Brennan S, Gonzalez JM, Suki WN. FK506-associated thrombotic microangiopathy: report of two cases and review of the literature. Transplantation 1999;67:539-44

17. Myers JN, Shabshab SF, Burton NA, Nathan SD. Successful use of cyclosporin in a lung transplant recipient with tacrolimus-associated hemolytic uremic syndrome. J Heart Lung Transplant 1999;18:1024-1026

18. Yango A, Morrissey P, Monaco A, Butera J, Gohh RY. Successful treatment of tacrolimus-associated thrombotic microangiopathy with sirolimus conversion and plasma exchange. Clin Nephrol 2002; 58:77-78

19. Tezcan H, Zimmer W, Fenstermaker R, Herzig GP, Schriber J. Severe cerebellar swelling and thrombotic thrombocytopenic purpura associated with FK506. Bone Marrow Transplant 1998;21:105-109

20. Burke GW, Ciancio G, Cirocco R, Markou M, Olson L, Contreras N, Roth D, Esquenazi V, Tzakis A, Miller J. Microangiopathy in kidney and simultaneous pancreas/kidney recipients treated with tacrolimus: evidence of endothelin and cytokine involvement. Transplantation 1999;68:13336-1342

21. Gharpure VS, Devine SM, Holland HK, Geller RB, O'Toole K, Wingard JR. Thrombotic thrombocytopenic purpura associated with FK506 following bone marrow transplantation 1995;16:715-6

22. Zarifan A, Meleg-Smith S, O'Donovan R, Tesi RJ, Batuman V. Cyclosporine-associated thrombotic microangiopathy in renal allografts. Kidney Int 1999;2457-2466

23. Pisoni R, Ruggenenti P, Remuzzi G. Drug-Induced thrombotic microangiopathy. Drug Saf 2001;24:491-501

24. Wiederrecht GJ, Sabers CJ, Brunn GJ, Martin MM, Dumont FJ, Abraham RT. Mechanism of action of rapamycin: new insights into the regulation of G1-phase progression in eukaryotic cells. Prog Cell Cycle Res 1995;1:53-71

25. Khanna AK. Mechanism of the combination immunosuppressive effects of rapamycin with either cyclosporine or tacrolimus. Transplantation 2000;70:690-694

26. Masterson R, Leikis V, Perkovic V, Nicholls K, Walker R. Sirolimus: a single center experience in combination with calcineurin inhibitors. Transplant Proc 2003;35(suppl 3):99S-104S

27. Hartwig T, Pridohl O, Witzigmann H, Lamesch P, Fangman J, Hauss J, Kohlhaw K. Low-dose sirolimus and Tacrolimus in kidney transplantationL first results of a single-center experience. Transplant Proc 2001;33:3226-3228

28. Langer RM, Van Buren CT, Katz SM, Kahan BD. De Novo hemolytic uremic syndrome after kidney transplantation in patients treated with cyclosporine-sirolimus combination. Transplantation 2002;73:756-760
29. Langer RM, Van Buren CT, Katz SM, Kahan BD. De Novo Hemolytic Uremic syndrome after kidney transplantation in patients treated with cyclosporine A Sirolimus combination. Transplant Proc 2001;33:3236-3237
30. Heering P, Deppe CE, Farokhzad F, Helmchen U, Grabensee B. Hemolytic uremic syndrome after renal transplantation: immunosuppressive therapy with rapamycin. Nephron 2002;91:177
31. McLeod BC, Thrombotic Microangiopathies in Bone Marrow and Organ Transplant Patients. Journal of Clinical Apheresis 2002;17:118
32. Meisenberg B, Sarkodee-Adoo C, Sotirescu D, Sensenbrenner L, Rapoport A,Cottler-Fox M, Tricot G, Ruehle K. Thrombotic microangiopathy in blood and marrow transplant patients receiving tacrolimus or cyclosporin A. Transfusion Medicine 2003; 43:78
33. Venkat KK, Karthikeyan V, Parasuraman R, Shah, V, Vera E. Outcome of Plasma Exchange Therapy in Thrombotic Microangiopathy After Renal Transplantation. American Journal of Transplantation 2003;3:1289
34. Litzow MR, Elliott MA, Nichols WL, Plumhoff EA, Ansell SM, Dispenzieri A, Gastineau DA, Gertz MA, Inwards DJ, Lacy MQ, Micalleff INM, Tefferi A. Post-transplantation Thrombotic Thrombocytopenia Purpura: A single-

Center Experience and a Contemporary Review. Mayo Clin Proc 2003;78:421

35. Lichtman MA, Hoyer LW, Sears DA. Erythrocyte deformation and hemolytic anemia coincident with the microvascular disease of rejecting renal homotransplants. Am J Med Sci 1968; 256:239.

36. Abbott KC, Yan CM, Agodoa LY, Reynolds JC. Thrombotic microangiopathy after renal transplantation in the United States. American Journal of Kidney Disease 2003;42: 1058 .

Table 2: Previously reported cases

Age	Gender	Organ transplanted	Time	FK506 dose	Treatment	OUTCOME	REFERENCE
36	F	KIDNEY	8	12MG/DAY	PEX, STOP TAC	FAVORABLE	SCHMIDT ET AL
27	F	BONE MARROW	3	0.15MG/KG	PEX, STOP TAC, HE	DEATH	ICHIHASHI T ET AL
32	F	LIVER	11	8MG/DAY	PEX, REDUCE TAC	FAVORABLE	HOLMAN ET AL
NCS	NCS	KIDNEY	1	0.1MG/KG	NCS	NCS	STUDY GROUP
36	F	KIDNEY	9	14MG/DAY	STOP TAC	NCS	RANDHAWA ET AL
29	F	KIDNEY	<1	20MG/DAY	REDUCE TAC	FAVORABLE	RANDHAWA ET AL
54	M	KIDNEY	1.5	45MG/DAY	REDUCE TAC	FAVORABLE	RANDHAWA ET AL
75	M	KIDNEY	<1	18MG/DAY	REDUCE TAC	FAVORABLE	RANDHAWA ET AL
47	F	KIDNEY	1.5	10MG/DAY	REDUCE TAC	FAVORABLE	RANDHAWA ET AL
51	F	KIDNEY	3.5	36MG/DAY	REDUCE TAC	FAVORABLE	RANDHAWA ET AL
76	M	KIDNEY	<1	20MG/DAY	REDUCE TAC	Loss of graft	RANDHAWA ET AL
21	M	KIDNEY	18	12MG/DAY	REDUCE TAC	FAVORABLE	RANDHAWA ET AL
33	F	KIDNEY	8	10MG/DAY	REDUCE TAC	FAVORABLE	RANDHAWA ET AL
60	F	KIDNEY	2.5	12MG/DAY	REDUCE TAC	FAVORABLE	RANDHAWA ET AL
53	M	HEART	19	12MG/DAY	PEX, STOP TAC	DEATH	MACH-PASCUAL ET AL
62	M	LIVER	4	NCS	PEX, STOP TAC	DEATH	MACH-PASCUAL ET AL

NCS	NCS	KIDNEY	NCS	0.15MG/KG	REDUCE TAC	FAVORABLE	KATARI S ET AL
NCS	NCS	KIDNEY	NCS	0.15MG/KG	REDUCE TAC	NCS	GABER ET AL
NCS	NCS	KIDNEY	NCS	NCS	REDUCE TAC	NCS	GABER ET AL
38	M	KIDNEY	14	8MG/DAY	PEX, WARF	FAVORABLE	TRIMARCHI ET AL
30	F	KIDNEY	14	10MG/DAY	STOP TAC, WARF	Loss of graft	TRIMARCHI ET AL
60	F	LUNG	NCS	12MG/DAY	STOP TAC	FAVORABLE	MYERS JN ET AL
55	F	KIDNEY	<1	0.15 MG/KG	PEX, STOP TAC	FAVORABLE	YANGO ET AL
28	M	BONE MARROW	1	NCS	NCS	DEATH	TEZCAN ET AL
19	F	BONE MARROW	<1	NCS	NCS	DEATH	TEZCAN ET AL
35	F	PANCREAS/KIDNEY	<1	1MG/DAY	PEX, STOP TAC	FAVORABLE	BURKE ET AL
36	F	PANCREAS/KIDNEY	<1	IMG/DAY	PEX, STOP TAC	FAVORABLE	BURKE ET AL
55	F	KIDNEY	<1	NCS	PE, STOP TAC	FAVORABLE	BURKE ET AL
22	M	KIDNEY	3	NCS	STOP TAC	FAVORABLE	ZARIFAN ET AL
27	M	KIDNEY	<1	NCS	NO CHANGE	FAVORABLE	ZARIFAN ET AL
38	W	KIDNEY	32	NCS	STOP TAC	DEATH	ZARIFAN ET AL
48	F	BONE MARROW	1.3	.03MG/KG/DAY	STOP TAC	FAVORABLE	GHARPURE ET AL
38	M	BONE MARROW	<1	.03MG/KG/DAY	STOP TAC	DEATH	GHARPURE ET AL

NCS: not clearly stated; favorable: No death, graft loss, or prolonged morbidity;

PEX: plasmapheresis; TAC: tacrolimus; HE: Heparin; WARF: warfarin

FIGURE 2

Changes in the patient's serum lactate dehydrogenase and platelet count during the course of her two admissions

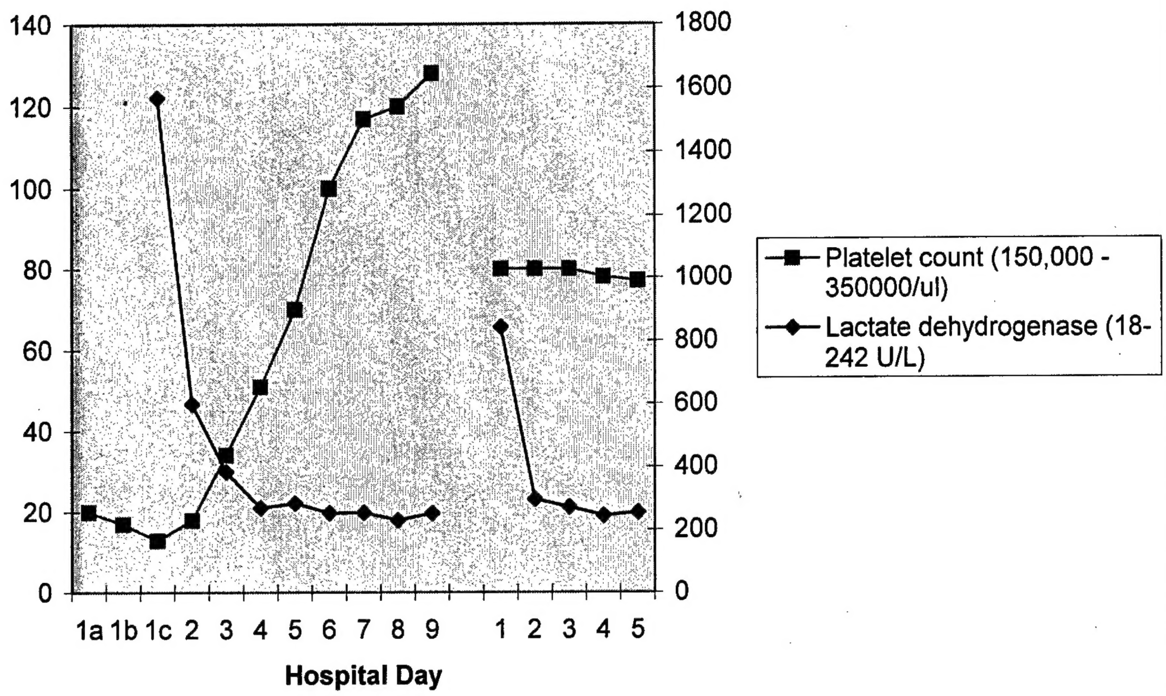

Table 1

TESTS/Hospital day	-4	1a	1b	1c	2	3	4	5	6	7	8	9		1	2	3	4	5
Hematocrit (37-47%)	32.6	30	28.2	25.2	27.9	27.8	25.1	26.3	26.4	27.5	27.7	27.2		31.9	26.9	28	31.4	29.5
Hemoglobin (12-16g/dl)	10.6	10	9.2	8.7	9.7	9.8	8.7	8.8	9.2	9.3	9.3	9		10.9	9	9	10.4	10.5
Platelet count (150,000 - 350000/ul)	230	20	17	13	18	34	51	70	100	117	120	128		80	80	80	78	77
Reticulocyte count (0.6-2.7%)					4.3			6.6			5.2			6.9	6.8			5.3
Lactate dehydrogenase (18-242 U/L)				1570	600	385	271	285	254	255	231	253		844	297	273	245	255
Bilirubin, total (<1.2 mg/dl)			2.04		1.57	1.1	0.6	0.51	0.37	0.34	0.36	0.29		1.26	0.84	0.69		0.21
Bilirubin, Direct (<0.2 mg/dl)			0.37		0.31	0.24	0.13	0.12	0.1	0.09	0.09	0.08		0.26	0.21	0.13		0.01

SGOT (0-35U/L)			97		55		40	68	60	53	68			57	25	25		28
SGPT (0-35U/L)			67		49		42	67	65	52	64			57	22	25		25
Blood Urea Nitrogen (8-18mg/dl)	16	16	17		12	14	12	7	12	11	8	12		15	14	13	12	11
Creatinine (0.5 - 1.2 mg/dl)	0.9	0.7	0.8		0.8	1.2	1.2	1	1.1	0.9	0.9	1		1	0.9	0.9	0.8	1
Potassium (5-5.0 mmol/L)	3.4	3.5	0.8		3.1	3.3	3.3	3.4	3.2	3	3.2	3.5		3.6	3.3	3.6	3.5	3.1

Table 1: Selected hematology and chemistry values during the patient's clinical course. The initial 9-day admission is shown on the right panel (1A) and the subsequent 5-day admission is shown on the left panel (1B). The top column represents a hospital day. The first column of values (header "-4") represents values obtained at a clinic visit 4 days prior to initial admission. Columns 1a-c represents 3 values obtained over a 4-hour period on the patient's day of admission.